INTERACTION OF THE BASIC COPOLYMER – POLY(ORNITHINE, LEUCINE) WITH NORMAL AND TRANSFORMED CELLS

Dan DUKSIN*, Ephraim KATCHALSKI-KATZIR and Leo SACHS
Departments of Biophysics and Genetics, The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

Transformed cells differ in many behavioral traits and biochemical parameters from the normal cells from which they were derived. It is generally accepted that the changes occurring in the structure of the plasma membrane represent rearrangement in the macromolecular structure of the cell surface. The introduction of lectins [1–5] into cancer research was a useful step in elucidating these structural differences as well as the mechanism of the malignant transformation. The lectins, however, were found to agglutinate most of the transformed and malignant cell lines. It was therefore important to find molecules which could react specifically with only one type of transformed cell, thus detecting changes that occur in one specific transformation.

We previously showed [6] that the synthetic random copolymer of ornithine and leucine-poly-(ornithine, leucine) (POL) can serve as such a tool. Although POL was found to rapidly agglutinate cells from all the cell lines tested in the absence of serum, it only caused cells transformed by the oncogenic virus SV40 to aggregate specifically, when incubated with the cells for 24 h in the presence of serum. This polypeptide was further found to be toxic to bacteria [7] and to mammalian cells [6], this toxic effect was used as a tool in the selection of resistant cell variants from SV40 transformed cells [8].

In this paper we present data on the uptake of a radioactively labeled POL by different cell lines, on the distribution of this peptide within these cells and the relation of this distribution to the in vitro effects.

* Present address: Department of Biochemistry, University of Washington, Seattle, WA 98195 USA

2. Experimental

2.1. *Cells and cell cultures*

The cell lines used in the present experiments were described previously [6]. Cells were maintained in Eagle's medium supplemented with a four-fold concentration of amino acids and vitamins (EM) and with 10% calf serum. The cells were grown in 60 mm plastic Petri dishes (NUNC) and kept in a humidified incubator at 37°C in a mixture of 90% air and 10% CO_2. All cells were routinely passaged with a Trypsin-Versene mixture solution every 3–4 days [6]. Routine mycoplasma tests were carried out on the cultures with the mycoplasma agar method [9].

2.2. *Polyamino Acids*

Radioactively-labeled poly(ornithine, leucine) (^{14}C-POL) was synthesized as previously described [6] using L-1-[^{14}C] leucine (Radiochemical Centre, Amersham, 62 mCi/mmol) and L-ornithine. The average mol. wt of ^{14}C-POL in aqueous solutions was 100 000 determined by sedimentation measurements and the specific radioactivity was 4.65×10^8 dpm per gram. Poly-L-glutamic acid (DP = 620) was kindly supplied by Mr I. Jacobson.

2.3. *Uptake of radioactive ^{14}C-POL by cells*

Two methods were used to examine POL uptake:

(a) Uptake of ^{14}C-POL by isolated washed cells: cells were suspended from the plates by trypsin or versene treatment, washed three times with phosphate buffered saline (PBS) and dispersed at a final concentration of 2×10^6 cells per ml in PBS. The suspension was then incubated for 20 min with various amounts of ^{14}C-POL (5–40 μg per ml), washed three times with PBS, dissolved in 0.1 N NaOH and counted in a scintillation counter in dioxane-based scintillant solution.
(b) Uptake of ^{14}C-POL by growing cells: cells from various cell lines were seeded (5×10^5 cells per plate) in 5 ml EM supplemented with 10% calf serum. ^{14}C-POL (100 μg) was added to the medium and the cells were incubated up to 24 h. At different intervals the medium was removed from 4 plates, the cell layer was washed three times with PBS, dissolved in 0.1 N NaOH and counted as described above. The volume of the cells for cell surface measurement was determined as previously described [10]. Protein was measured using bovine serum albumin as standard [11]. When removal of absorbed ^{14}C-POL was attempted cells were incubated in PBS alone or in PBS containing polyglutamic acid (20 μg per ml) for an additional period of 20 min after the third wash with PBS. Following this incubation the cells were washed twice with PBS and counted as described above.

2.4. *Treatment of cells with Nonidet P-40*

Growing cells from the different cell lines were incubated for 2–24 h with ^{14}C-POL in Eagle medium containing 10% calf serum. At the end of the incubation period the plates were washed three times with PBS and 2 ml of a PBS solution containing 0.1% Nonidet P-40 were added to each plate. The plates were further incubated for 10 min at 4°C. This treatment was shown to solubilize the cell-cytoplasm leaving the intact nuclei attached to the plates [12,13]. The cytoplasmic fraction was collected separately, whereas the attached nuclei were washed three times with PBS and harvested using a rubber policeman dissolved in 0.1 N NaOH and counted separately from the cytoplasmic fraction. Microscopic observations in a phase-contrast field and cell counts showed that the different cell lines reacted similarly to the treatment with Nonidet P-40, i.e., the cell-cytoplasm was completely dissolved whereas the nuclei remained intact.

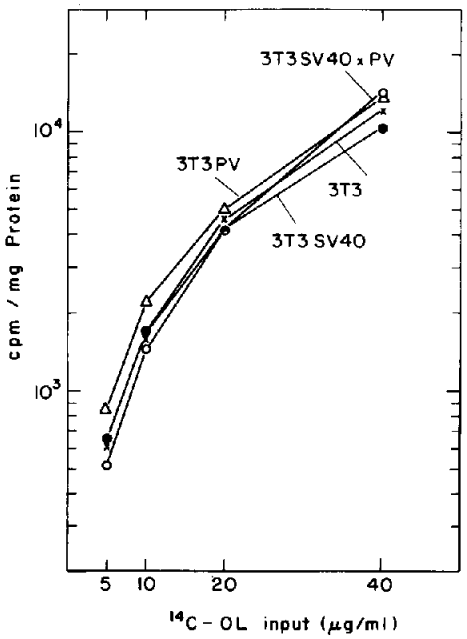

Fig.1. The adsorption of ^{14}C-POL by different cell lines calculated as related to cell protein. The cells were washed by PBS and incubated with ^{14}C-POL in PBS for 20 min, washed 3 times with PBS and counted.

3. Results

3.1. *Uptake of ^{14}C-POL by washed cells*

The uptake of ^{14}C-POL was measured with cells derived from the 3T3 cell line: untransformed 3T3 cells, 3T3 cells transformed by polyoma virus (3T3PV), 3T3 cells transformed by SV40 (3T3SV40) and 3T3 transformed by both viruses (3T3 SV40 PV). The uptake of ^{14}C-POL by washed cells calculated as cpm per mg cell protein is shown in fig.1. The figure shows that the uptake of ^{14}C-POL by all four cell lines is similar. Similar results were obtained with hamster cells (hamster fibroblasts, hamster SV40 transformed, polyma transformed and dimethylnitrosamine transformed cell lines). When calculated as number of molecules per square micrometre surface area of cell membrane approx. 10^4 molecules were adsorbed per μm^2 cell surface at an input of 10 μg per ml ^{14}C-POL.

3.2. *Uptake of ^{14}C-POL by growing cells*

POL causes rapid agglutination of washed cells.

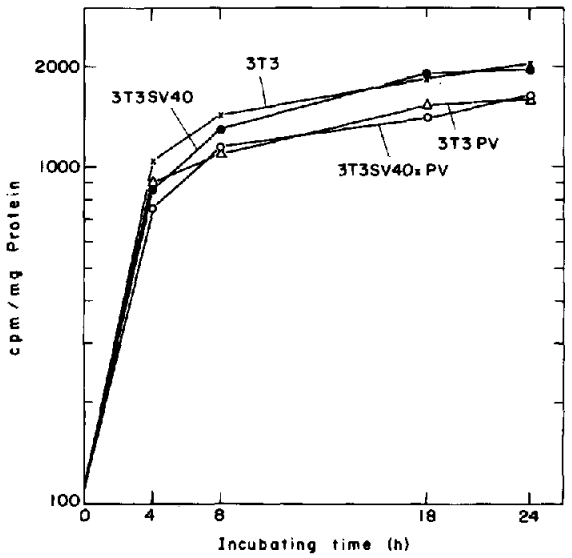

Fig. 2. The adsorption of ^{14}C-POL by different cell lines incubated with POL in Eagle's medium supplemented with calf serum, calculated as related to cell protein. The cells were incubated in the presence of ^{14}C-POL (20 μg per ml) up to 24 h. Samples of cells were harvested at different time intervals, washed 3 times with PBS and counte

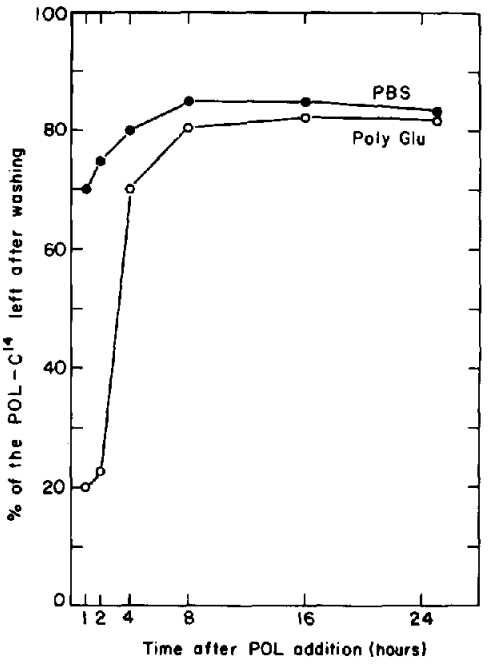

Fig. 3. The treatment of adsorbed ^{14}C-POL to 3T3SV40 cells by PBS and by polyglutamic acid. 20 μg ^{14}C-POL per ml were incubated with 3T3SV40 cells for 24 h in Eagle Medium containing 10% calf serum. At different time intervals samples of cells were washed 3 times with PBS and incubated for an additional 20 min with PBS only or PBS containing polyglutamic acid (20 μg per ml, DP=620), washed and counted.

However, only SV40 transformed cells undergo specific aggregation when incubated with POL in the presence of serum. To examine this phenomenon further the uptake of ^{14}C-POL by growing cells in the presence of serum was determined. Fig. 2 shows uptake of ^{14}C-POL by growing cells. Again, the different cell lines adsorbed similar amounts of ^{14}C-POL; specifically, no difference was found between the uptake of the peptide by SV40 transformed cells and the uptake by the corresponding normal or polyoma transformed 3T3 cells.

Previous studies showed that the specific aggregation of SV40 transformed cells, caused by POL, could be prevented by addition of polyacidic amino acids such as polyglutamic acid. The prevention of the aggregation was possible only if polyglutamic acid was added together with, or up to 5 h after, POL addition [6]. The ability of polyglutamic acid to remove ^{14}C-POL adsorbed to cells is presented in fig. 3. When polyglutamic acid was added during the first four hours after POL addition, most of the ^{14}C-POL (80%) is removed whereas only 20–25% of the radioactivity is removed by PBS. When cells were incubated with radioactive POL for longer periods, only a small proportion of the POL (15–20%) is removed by polyglutamic acid.

3.3. *Differences in the distribution of ^{14}C-POL in SV40 transformed cells*

The different cell lines were incubated with ^{14}C-POL as described above. The cells were washed and then incubated with the detergent Nonidet P-40. This detergent destroys the outer cell membrane without disrupting the nuclear membrane and allows for the separation of cell nuclei from cytoplasm. As demonstrated in fig. 4 most of the radioactive POL (80% after 24 h of incubation) in the SV40 transformed cells was found in the cytoplasmic fraction and only a small portion of the label was found in the nuclear

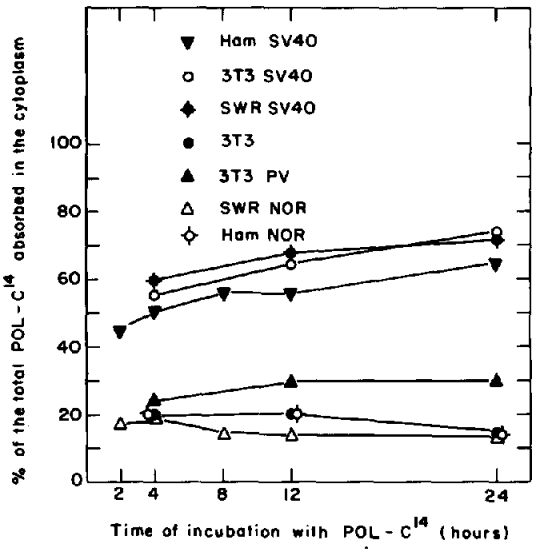

Fig.4. The percentage of ^{14}C-POL found in the cytoplasm of different cell lines. 20 μg per ml ^{14}C-POL were incubated with the different cell lines for 24 h in Eagle Medium containing 10% calf serum. At different time intervals the cells were washed 3 times with PBS and incubated for 10 min in PBS containing 0.1% Nonidet P-40. Cytoplasmic and nucleic fraction were separated and counted separately.

fraction. Table 1 represents the specific radioactivity (cpm ^{14}C-POL/mg protein) in the cytoplasmic fraction of 3T3 SV40 cells that was found to be 5 times higher than that of 3T3 cells and 3 times higher than that of 3T3 polyoma virus transformed cells. Fig.4 also shows that in all the other cell lines tested, most of the ^{14}C-POL (80%) was found in the nuclear fraction. Reconstruction experiments, in which labeled nuclear fractions were mixed with unlabeled cytoplasmic fractions and vice versa, showed that the exchange of radioactivity between the fractions is less than 3% (not shown).

4. Discussion

The specific aggregation of transformed cells by POL in the presence of serum appears to be a consequence of infection of those cells with SV40 virion but not due to specific adsorption of the peptide to SV40 transformed cells. In similar conditions the different cell lines adsorbed the same amounts of ^{14}C-POL. Similar observations were found with Con A [10] and with soybean agglutinin [14].

Polyglutamic acid was previously shown to inhibit the specific aggregation of SV40 transformed cells caused by POL, but only if added during the first few hours after POL addition [6]. The addition of polyglutamic acid during this time removes most of the adsorbed ^{14}C-POL; addition at later times has no effect. This appears to be due to penetration of POL into the cells so that it is no longer available for removal by polyglutamic acid. Basic polymers are known to increase membrane movement leading to increased phagocytosis and endocytosis in animal cells [15] which suggests that POL is included in cells by such a

Table 1
Adsorption of ^{14}C-POL by 3T3 cell lines and the distribution of the peptide in cytoplasmic fraction

Cells	^{14}C-POL adsorbed (cpm)	^{14}C-POL in cytoplasmic fraction (cpm)	Cell protein mg	Cytoplasmic fraction protein mg	Specific radioactivity (cpm/mg Prot)
3T3	4720	708	0.87	0.68	1050
3T3SV40	4940	3956	1.03	0.71	5600
3T3PV	5320	1600	1.20	0.89	1790

The cells were incubated for 24 h in the presence of serum and ^{14}C-POL. After the incubation period the cells were washed and incubated with 0.1% Nonidet P-40 as described in Experimental. Cytoplasmic and nuclear fractions were separated and counted separately. The numbers given are the average of 3 experiments.

mechanism. Perhaps of greater significance is the finding that ^{14}C-POL was found in the nuclear fraction of the cells and the difference in distribution of ^{14}C-POL between the nuclear and cytoplasmic fractions in the different cell lines. In SV40 transformed cells incubated with POL, about 70% of the peptide remained in the cytoplasm fractions whereas in the other lines the peptide was concentrated in the nuclear fraction. The function of this distribution in producing specific aggregation is to be further studied.

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